BROWN SWISS WEAVER SYNDROME: STUDIES OF MUSCLE PATHOLOGY

by

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B.S., Kansas State University, 1978

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1980

Approved by:

Major Professor

DEDICATION

Dedicated to my mother,
Dorothy C. Mueller, for
her continual encouragement.

ACKNOWLEDGEMENTS

I wish to thank Professor H. W. Leipold for his guidance, encouragement and assistance throughout my course of study and research. I am also grateful to Professors S. M. Dennis and S. M. Kruckenberg for their critical reading of the thesis and advice. I am also especially grateful to my entire family who has persevered with me through my years of schooling. Finally, to my wife, Mary Jo, and son, John, my thanks for their love and understanding during this project.

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INTRODUCTION

The direct relationship between congenital and hereditary myopathies and economics justify examination of causes and prevention where possible. Congenital defects are abnormalities of structure or function present at birth (Leipold et al, 1972). In spite of the fact that a defect is present at birth, it may not be identifiable until later in life and requiring clinical, biochemical or pathological examination for confirmation.

The lack of a universally accepted system for classifying congenital defects makes comparison of reported data difficult, since myopathies are often reported as musculoskeletal or muscle-skin defects. Nevertheless, all myopathies are of economic importance and frequent occurrence. A nine year study of 2,293 congenitally affected calves in Hesse, Germany, assigned a value of 13.7% to the frequency of musculature defects (Rieck, 1968). Greene et al (1973) reported a frequency of 14.9% for muscle defects of calves in Kansas. The frequency of any individual defect or body system defect, and the total number of defects likely, will vary with breed, geographic area, and year (Leipold et al, 1972). After examining the above data the significance of congenital myopathies to the

animal production industry becomes more apparent, as muscle is sold as food.

Congenital defects result from genetic and environmental factors or a combination of both (Leipold et al, 1972). Genetic factors are characterized either by chromosomal aberrations, which can be directly diagnosed, or by mutant genes, which can be identified indirectly by their pattern of transmission (Saperstein et al, 1975). Except for chromosomal aberrations, genetic defects are recognized only when they occur in characteristic intragenerational familial frequencies and intergenerational patterns (Leipold et al, 1972). All genetic defects have environmentally-induced phenocopies which should be kept in mind when defining causative agents. Viruses, drug and plant teratogens, age, hypoxia, hyperthemia, season and nutrition have been cited as environmental factors causing congenital defects. Unfortunately, specific teratogens or their signs often cannot be demonstrated or isolated even after extensive pathological investigations (Leipold 1978a). It is with these thoughts in mind that a review of congenital myopathies in animals was undertaken.

I. REVIEW OF LITERATURE

Myopathies in Cattle

Congenital Flexure of Pasterns

Congenital flexure of pasterns is characterized by knuckling over of the fore pasterns and is known to occur in calves, lambs and foals. It is bilateral although it may be asymmetrical. The fore limbs are always involved with the hind limbs occasionally affected (Jubb and Kennedy 1970). Mead et al (1943) and Dale and Moxley (1952) documented the condition in the Jersey and Milking Shorthorn breeds, respectively, and incriminated a recessive mode of inheritance. Jubb and Kennedy (1970) reported occurrence in other European and Oriental cattle, but added that there were cases where there was no genetic involvement. All calves affected display the same phenotypic appearance and patterns. The defect is due to developmental immaturity of the flexor muscles and recovery of affected animals occurs 1 to 8 weeks after birth depending upon initial severity. This myopathy, although not permanently disabling, is common and affects growth and increases age at maturity, in severe cases, may result in dystocia and/or fetotomy.

Limber Leg

Limber leg is a descriptive term for a congenital limb defect in Jersey calves that lack muscle control (Greene et al, 1973). Characteristic clinical signs are abnormal flexure of joints, especially the shoulder, elbow, knee, hip, stifle and hock. Generally, all four legs are affected. Calves are normal in outward appearance, but they have little or no control over movement of their legs and are unable to stand (Lamb et al, 1976). The calves are able to eat and may live for several weeks, but unlike congenital flexure of the pasterns, the condition does not improve with time, necessitating their destruction.

Gross pathological lesions are confined to musculature and joints of the legs. The muscles have varying degrees of atrophy from moderate to severe. The articular surfaces display either shallowness with rounded outlines or unevenness and distortion. Histopathological findings are characterized by shrinking of sarcoplasm, partially empty sarcolemmal tubules and concentrated sarcolemmal nuclei due to reduction of muscle mass (Greene et al, 1973).

The mode of inheritance of this myopathy was determined by Lamb et al (1976) in a study of 105 cases of limber leg in Jersey calves. They concluded that the semilethal defect was inherited as a simple autosomal recessive factor. The increase in incidence was due to popularity and widespread

use of a carrier bull, Favorite Commando, his daughter, Marlu Milady and many of their descendants. The American Jersey Cattle Club has taken steps to eliminate this defect.

Muscular Dystrophy

Adams (1975) defined muscular dystrophy as a hereditary, progressive, degenerative disease of muscles. Muscular dystrophy has been described in sheep by Hartley and Dodd (1957) and McGavin (1974). Lambs are unable to rise after birth and die under range conditions. McGavin has maintained affected sheep under experimental conditions for several years. The pathological lesions are: variation of fiber diameter, peripheral sarcoplasmic masses and numerous muscle nuclei, some vesicular and centrally located. There was evidence of ongoing replacement of muscle fibers by fat with no detectable central nervous system defects.

Leipold et al (1973) described a muscular dystrophy in cattle called weaver syndrome in the Brown Swiss breed. The affected cattle were usually not detected until 6 to 8 months of age when they displayed a peculiar, erratic gait and stance with the fore legs spread far apart while the hind legs are almost together. Clinical and histopathological examination revealed no neurologic disorder. Sections of quadriceps muscle revealed a lack of regeneration

with muscle replacement by adipose tissue. The mode of inheritance has not been delineated.

Muscular Hypertrophy

Muscular hypertrophy (double muscling) has been well documented as occurring in all breeds of cattle (Lauvergne et al, 1963; Butterfield 1966; Oliver and Cartwright 1969; Jubb and Kennedy 1970; Leipold et al, 1972; Bradley 1978; Rwuaan 1979). In contrast, the condition has only been reported once in sheep (Dennis 1972b).

In cattle, the myopathy is inherited, causing an enlargement of certain muscles, particularly those of the loin and hind quarter. The mode of inheritance, although the topic of some debate, has been determined by Oliver and Cartwright (1969) and confirmed by Rwuaan (1979) as being incompletely recessive with variable expressivity. This variability accounts for the difficulty in determining heterozygotes by their phenotypes. Oliver and Cartwright (1969) classified the cattle population into three categories: Group one contains animals with a normal phenotype; the second group includes those with most or all of the characteristics of double muscling to an extreme degree; and the third group contains animals with some of the typical characteristics of double muscling. This last group has animals that are quite variable in the number of

characteristics they exhibit and the intensity or degree to which they display the characteristic. In determining placement into a particular group, Oliver and Cartwright (1969) used the French system of ten characteristics: hypertrophy of the tongue, muscular hypertrophy of the shoulders, body width in relation to height, reduction of the pauch, inclination of the rump, tail attachment, muscular hypertrophy of the thighs, impression of the superficial grooves between muscles, position of the legs, and fineness of the bones of the fore legs. Each animal was given a subjective score on each category, 0 if normal, 1 if intermediate, and 2 if double muscled. Any animal scoring more than 6 points was considered double muscled.

Determining carrier cattle is important to breeders who wish to promote this type of animal or to eliminate the double muscled individual. Advantages of double muscling outlined by Bradley (1978) were: thin hide giving finer leather, much less subcutaneous fat, fine-boned skeleton with advantageous muscle to bone ratio, relative increase in size of muscles of greatest importance, and double muscling produces more juicy and tender meat. Disadvantages detailed by Bradley were: thin hide leaves animal injury prone, deficiency of subcutaneous fat provides poor insulation for heat conservation, finer bones

and greater body weight put more strain on smaller bones and joints, rickets is more common, small visceral weight increases stress susceptibility, decreased viability of calves, increase in dark cutters, reduced fertility, dystocia, increased embryonic loss, reduced lactation, and macroglossia. The demand for more meat per animal unit by consumers must not influence animal breeders into promoting propagation of a known defect. Rwuaan (1979) concluded that cattle suspected of carrying the double muscling trait should not be used for breeding.

Arthrogryposis

Arthrogryposis neither implies a specific pathologic entity nor a specific cause (Leipold 1978a). Defined as permanent, abnormal joint fixation present at birth, arthrogryposis is one of the most frequent congenital defects observed in calves. The condition has also been described in man, foals, pigs and lambs (Leipold et al, 1970). The syndrome is known in all breeds of cattle with a worldwide distribution. Dennis and Leipold (1979) reported arthrogryposis was common in lambs. The syndrome has a diverse etiology in all species. Much of the work to date has been related to the genetics of the syndrome.

Several reports in cattle proposed a genetic cause (Leipold et al, 1969, 1970, 1972; Leipold 1978a,b; Hadlow

1973; Goonewardene and Berg 1976; Hanset et al, 1978) but viruses (Nobel et al, 1971; Shepard et al, 1978; Shimshony 1978; St. George et al, 1978) and phytoteratogens (Shupe et al, 1967; Young 1969; Hamada 1974; Young and Blair 1974; Leipold 1978b) have also been incriminated as causative factors of arthrogryposis. Unknown causes have been reported in lambs by Dennis (1974) and in cattle by Leipold et al (1972). Associated defects reported included: cleft palate, spina bifida, microencephaly, ancephaly, atresia ani, kyphoscolisis, hydromyelia, and syringomyelia.

Genetic recessive inheritance with modified penetrance has been documented in Charolais by Leipold et al (1969) and further research has confirmed their original findings. Leipold et al (1969) described arthrogryposis in Charolais often associated with defects of cleft palate and hypoplasia of the patella along with spinal dysraphism. The genetic form has muscle wasting and widespread replacement of muscle fibers by fat. Hadlow (1973) reviewed genetic arthrogryposis in the Welsh Mountain and Australian Merino breeds of sheep and described inheritance as an autosomal recessive trait. Limb muscles were pale, hypoplastic and sometimes fibrous. Histological examination revealed fibers lacking striation and replacement of muscle fibers by fat.

Viral causes have been studied in lambs by many investigators (Nobel et al, 1971; Hartley and Haughey

1974; Rudert 1978; Shimshony 1978) and in calves (Young 1969; Hamada 1974; Young and Blair, 1974; Shepard et al, 1978; St. George et al, 1978). The viral agent indicated was akabane virus with the insect vector being Culicoides brevitarsis. There has been mention of other possible insect vectors, but none were named specifically. Research in this area is continuing as evidenced by current emphasis in the literature.

Shupe et al (1967) have described plant induced arthrogryposis. The phytoteratogens indicated was Lupinus serceus and Lupinus candatus. The plants must be ingested by cows between day 40 and 70 of gestation. The time table is proportionately adjusted for pregnant ewes. The authors recommended that pregnant animals be withheld from pastures for that period and the problem would be alleviated.

In light of the current emphasis on nongenetic agents causing arthrogryposis, reports of unknown causes in cattle and sheep could be explained as result of an environmental or genetic-environmental causes.

Nutritional Myopathies

The most common and therefore the most economically significant myopathy in domestic ruminants is nutritional in origin. Young animals, particularly those of suckling age, are affected. Nutritional myopathies have been reported

in many parts of the world are known to occur enzootically in countries of temperate climate (Amstutz 1964; Donaldson 1970; Jubb and Kennedy 1970; Hadlow 1973; Garden and Sproat 1978; Allen and Friend 1978; Leipold 1978a; Dennis and Leipold 1979). Sheep and cattle display common clinical signs: rapidly developing symmetrical weakness of hind quarter muscles resulting in a stiff gait, difficulty in standing leading to inability to stand, prostration and death within a week after onset. Inanition and broncho-pneumonia are often responsible for death. Acute cardiac failure is a particular feature of the disease in calves (Hadlow 1973). Cardiac lesions in calves are located in the left ventricle and septum, whereas in lambs, the lesions are located in the right ventricular wall.

The gross muscle lesions of nutritional myopathy or white muscle disease (WMD) varies from diffuse colorless areas or white to gray-yellow streaks to large well defined patches with a cooked appearance. Microscopically, hyaline degeneration of entire fibers is the characteristic change. Fine calcified areas are found arranged along myofibrils. Prominent proliferation of muscle nuclei is a common feature indicating attempts at regeneration that may be well advanced in animals that die suddenly (Hadlow 1973; Jubb and Kennedy 1970; Thomson 1978).

Factors involved in WMD in cattle and sheep are: vitamin E deficiency, selenium deficiency, presence of legumes in the diet, presence of polyunsaturated fatty acids (fish oil) in the diet, stress, and unaccustomed exercise. Garden and Sproat (1978) along with Rogers and Poole (1978) cited a possibility of an inherited predisposition to nutritional myopathy in cattle. Although there are no reports of this predisposition in sheep, it is a possibility.

In cattle, two clinical forms of white muscle disease are recognized. Acute cardiac form that predominates in young calves often results in death a few hours after onset of labored breathing, bloody nasal discharge and weakness. The skeletal muscle form is varied in expression and develops more slowly than the cardiac form. Mildly affected calves often recover after treatment and rest. The latter form is often referred to as exercise myopathy, being precipitated by sudden exercise or stress. Dietary supplementation with vitamin E and selenium will often alleviate the condition.

WMD or stiff lamb disease in sheep is similar to that in cattle with the two forms being evident. However, the skeletal muscle form predominates. Affected lambs are unable to move or suckle and swallowing may be difficult.

Recovery is common with vitamin E or selenium treatment; however failure to treat often results in a 40 to 50 per cent mortality.

WMD is an important and complicated problem for the livestock industry, and requires more research into the biochemical and environmental relationships involved.

Myopathies in Swine

Myopathies of swine are as economically important as those in cattle and sheep. In fact, swine myopathies may have greater economic impact due to the higher number of offspring per pregnancy and decreased generation interval. Although cattle, sheep and swine share certain myopathies, such as arthrogryposis, there are several significant myopathies that are known only in swine.

Arthrogryposis

Arthrogryposis in pigs is characterized by an over-flexion of limbs present at birth. The limbs also may be rigid, entwined over the body or hyperextended at the hock. The syndrome in swine is known to be genetically recessive and has been produced by ingestion of tobacco stalks (Nicotiana tabacum), jimson weed (Datura stramonium), poison hemlock (Cranium maculatum), wild black cherries

(Prunus serotina) or methallibure (Akpokodje and Barber 1971; Selby et al, 1971; Edmonds et al, 1972; Leipold et al, 1973; Crowe and Ward 1973; Swatland 1974; Bradley 1978; Huston et al, 1978). Occurrence is common, with a number of pigs in a litter being affected. Depending on severity and associated defects, affected pigs may recover. Associated defects reported included: brachygnathia, cleft palate, kyphosis, atresia ani, small external sex organs, and skull deformities. Associated defects in methallibure-induced arthrogryposis were: protrusion of liver through a diaphragmatic hernia, thin skin on roof of the skull, and pitted kidneys.

Splayleg

Splayleg is a condition in which the hind limbs, and sometimes the fore limbs, of a piglet are unable to support the body (Dobson 1968; Thurley and Done 1969; Patterson and Allen 1972; Swatland 1974; Bradley 1978; Huston et al, 1978). Affected limbs are splayed outwardly or forwards due to an apparent impairment of adductor ability. Affected pigs may recover spontaneously in 3 or 4 days if they are able to suckle and avoid being crushed by their sow. Providing nonslippery floors and fixing the hind limbs together at the level of the metatarsus with adhesive tape has been recommended to aid recovery of affected pigs (Bradley 1978).

Although this syndrome is essentially clinical in nature, several causative factors comprise the pathology of the myopathy: hereditary with sex-linked dominant inheritance; traumatic due to slippery floors; nutritional due to methionine and choline deficiencies; toxic due to Fusarium F_2 toxin in the diet of the pregnant sow; dysmaturity of skeletal muscle; developmental and myopathic with intensity of clinical signs dependent upon environmental factors. The current theory is a strong genetic component with which single or multiple environmental factors interact variably (Bradley 1978). Unfortunately, previous pathological and etiological investigations revealed nothing of pathogenetic significance.

Porcine Stress Syndrome

Porcine stress syndrome (PSS) and its accompanying pale, soft and exudative musculature is evident in approximately 20 per cent of all swine slaughtered and is associated with lower processing yields, increased cooking losses and reduced juiciness (Forrest et al, 1975). Pigs with this myopathy exhibit extreme muscularity, anxious behavior, muscle tremors, and erythema. After stress or an anesthetic agent, the affected pig has signs of rapid tremor in the tail, dyspnea, hyperthermia, erythema and blanching of the skin. Muscles become rigid and the pig is reluctant to

move. Death often occurs 4-6 minutes after onset of stress (Swatland 1974; Bradley 1978; Huston et al, 1978). Huston et al (1978) described the cause of PSS as a deficiency of monoamine oxidase and/or catechol ortho-methyl transferase after high amounts of norepinephrine which is usually caused by stress or anesthetic agents. A genetic predisposition or genetic cause has also been mentioned (Bradley 1978; Huston et al, 1978). Clinical testing of suspect breeding animals during stress and selective breeding of nonstress susceptible pigs will aid in controlling this economically important myopathy.

Asymmetric Hind Quarter Syndrome

Asymmetric hind quarter syndrome is a descriptive term for a myopathy that results in muscles of one thigh being especially reduced. Diagnosis can be made by clinical examination of pigs 2 or 3 months old. The asymmetry of hind quarters becomes more apparent as pigs approach slaughter weight. Frequency is rather low, but significant in herds and breeds in which the syndrome is expressed. Although several studies have been conducted (Done et al, 1975; Bradley 1978) the results were tentative suggesting genetic liability to develop the syndrome in the Large White, Hampshire, Lacombe and their crossbreds.

Asymmetry is due to an increase of subcutaneous fat on the larger side and to a relative increase in weight and volume of the adductor and posterior thigh muscles (Bradley 1978). The M. semitendinosis is usually the most affected, with the quadriceps remaining unaffected. Asymmetry of individual muscles is primarily due to reduction in number of muscle cells in the smaller muscle. Histologically, there is degenerative, atrophic or inflammatory changes (Done et al, 1975; Swatland 1974; Bradley 1978). Bradley (1978) attributed the asymmetry to peculiarities of fat distribution and abnormal distribution of muscle cells in asymmetric muscles, the larger muscle having more cells than the smaller, but with the same proportions of cell types as each other,as the same muscles from symmetrical pigs. Further research is being conducted in England.

Pietrain Creeper Syndrome

Pietrain pigs were introduced to England in 1964 to evaluate their genetic potential. Although few purebred Pietrain pigs remain in England, there are many commercial crossbreeds and sire lines with Pietrain heritage.

Pietrain creeper syndrome is recognized as a clinico-pathological entity in only one purebred herd in which selection for susceptibility to porcine stress syndrome had been practiced (Bradley 1978). The clinical signs were

trembling of the fore and hind quarters when standing and a marked reluctance to stand for any length of time. These signs gradually progressed to recumbency and deterioration in general condition. Death often occurred by age 12 weeks. There were no purely nervous signs, significant lesions being confined to skeletal musculature and increase in severity with the duration and intensity of clinical signs. Most muscles were involved in this myopathy. Lesions included muscle atrophy, characterized by internal nuclei, focal myodegeneration and regeneration in excess of normal Pietrain muscle.

Muscular Steatosis

Although muscular steatosis is rare in cattle and sheep, it is a common finding in slaughtered pigs (Jubb and Kennedy 1970; Hadlow 1973). The myopathy is characterized by progressive replacement of muscle by adipose tissue without changing the gross form of the affected muscles. Skeletal muscles, often all of them, are symmetrically affected. The most common muscle masses affected are those of the loin and hind quarter. Involvement is partial, beginning at the muscle's periphery, and irregularly progresses into the muscle. In cross-section, affected muscle has a coarsely marbled appearance. Histologically, the muscles may be atrophic or have

hyaline or vacuolar degeneration. The endomysium and perimysium are normal, although Jubb and Kennedy (1970) proposed that the lesion may terminate as sclerosis of the affected muscle. This myopathy has been proposed as having a genetic nature with modifying factors entering into its expression.

Nutritional Myopathy

Jubb and Kennedy (1970) and Hadlow (1973) reviewed nutritional myopathy in swine, treating it as a distinct entity related to vitamin E and/or selenium with strong possibilities of genetic predisposition being involved. The disease is similar to nutritional myopathy of cattle and sheep, and primarily affects rapidly growing pigs weighing 30 to 60 kilos. The myopathy has, however, been reported in newborn pigs and sows at parturition. The disease seems to be limited to Scandinavian countries and occurs in the fall after the pigs have been fed newly harvested grains. It is thought that excessive rainfall during the summer growing season enhances the myopathy-producing properties of the grain. Clinical signs mirror those in other farm animals. Two most prominent clinical signs are: a staggering gait, affecting the hind legs, and failure to rise from recumbency. Microscopic lesions are similar to those in cattle and sheep. Treatment with

vitamin E and/or selenium was effective in Sweden, which supports the nutritional basis for this myopathy.

Myopathies in the Horse

Myopathies in horses closely resemble those in other domestic animals, although they are less well defined. There is no explanation to be found for the lack of definition in the literature.

Contracted foal is a myopathy of the horse which is similar to arthrogryposis. The syndrome is not uncommon as approximately 60 cases have been documented (Rooney 1966, 1969; Finocchio 1973; Adams 1975; Irwin and Pulley 1975; Gunn 1976). The cause suggested by Rooney (1969) and Adams (1975) was malposition of the fetus in utero, but other authors indicate viral, environmental as well as genetic factors (Irwin and Pulley 1975; Gunn 1976). The pathological process involved in the related muscles vary. Rooney (1966) did not find any significant gross or microscopic lesions in the skeletal muscles of foals he examined. On the other hand, Gunn (1976) found hypoplasia of muscle fibers concomitant with an increase in adjacent fibrous tissue. The absence of any significant alteration in distribution of fiber types using the myosin ATPase reaction in affected muscles was a factor in Gunn's suggestion of a

myopathic rather than a neurogenic arthrogryposis in the foals he examined.

Hadlow (1973) reviewed nutritional myopathies in foals which resembled those of calves and lambs. The occurrence is common with many areas of the world reporting the myopathy. The clinical pattern varies with severity of the disease; the acute disease resulting in depression, stiffness and death within 1 to 7 days after onset. The other form is more gradual in nature with stiffness and often paralysis of masticatory muscles resulting in an inability to suckle. Less severe cases have been known to recover spontaneously (Hadlow 1973). Gross changes were: thickened, firm, yellowish-brown fat throughout the musculature, especially in the neck region. Kroneman and Wensvoort (1968) characterized the microscopic lesions in the altered fat as being edematous with massive infiltration of neutrophils and necrosis and calcification of fat cells. They also mentioned finding areas of acid fast pigment in affected Shetland foals, but no further conclusions were stated.

There has been supposition, but no hard proof that vitamin E and/or selenium is somehow related to this myopathy in foals. Hadlow (1973) mentioned that treatment of affected foals with vitamin E had disappointing results.

Myopathies in Dogs and Cats

Primary diseases of muscle are rare in the dog and cat. Wentik et al (1972) described a generalized myopathy in 5 male Irish Terriers in a litter of 8. The clinical signs at 8 weeks of age were: stiffness of gait, enlarged tongue, and atrophic muscles. As male puppies from two previous all male litters from the same bitch had the same signs, the muscular dystrophy was suggestive of an X-linked recessive mode of inheritance.

Primary progressive muscular dystrophy in the dog is a rare disease first reported by Innes (1951) in a single case report. Whitney (1958) reported three cases of a limb girdle type dystrophy. Funkquist et al (1980) reported a case in a Labrador dog. Although there were few reports, the histopathological lesions were remarkably similar; the primary features being a marked variation in fiber size and fat replacement of atrophic muscle fibers. No regeneration was evident (Whitney 1958; Funkquist et al, 1980). Nerves examined were normal.

Genetic factors causing muscular dystrophy are well known. It is possible that there is a genetic basis for the disease in dogs as well, due to the fact that the majority of cases reported involve Labrador dogs.

Meier (1958) found polymyositis in 2 of 3,000 dogs he necropsied, but the cause was not apparent. Both dogs displayed stiffness of gait with progressive atrophy resulting in recumbency. Microscopically, there was extensive hyaline degeneration accompanied by prominent proliferation of muscle nuclei in the wasted muscles. Many lymphocytes and neutrophils were present in some areas, but a widespread inflammatory response was absent.

Hadlow (1973) reviewed an example of polymyopathy in a Collie sheepdog that was characterized by bilateral, symmetrical, pale areas in all the main muscles. The appearance of the muscles resembled nutritional myopathy in cattle and sheep. This similarity was explained by the dog's diet that consisted mainly of selenium-deficient mutton.

Gardner (1967) described a nutritional myopathy in a cat whose diet consisted of raw mutton and milk. Swelling, coagulation necrosis, fragmentation and calcification of muscle fibers were the histopathological findings. Grossly, pale, bilateral symmetrical muscle masses were present. Selenium deficiency was the probable cause of this myopathy.

Myotonia in Chow Chow dogs was reported by Jones et al (1977). Stiffness of gait, muscle spasms and dystrophic changes of biopsied muscles were reported. Diagnosis of myotonia was confirmed by electromyography. The authors

suspected an inherited cause for this myopathy as all affected dogs were related.

Smythe (1970) reported a Toy Poodle with developmental fibrosis of the plantarsis muscle that resulted in inability to flex the tarsus. The affected puppies hopped instead of walking. This condition was corrected surgically. The surgical procedure must be performed as soon after weaning as possible to insure successful recovery. The mode of inheritance of this myopathy is unknown, although occurrence in the breed is common.

An inherited storage disease of cats, characterized by progressive muscle weakness and atrophy, was described by Hegreberg and Norby (1973). The disease is inherited as a simple autosomal recessive trait and results in death between 1 and 4 months of age. Muscle changes included focal segmental coagulation necrosis, proliferation of sarcolemmal nuclei, and random variation in size of muscle fibers within muscle bundles. The frequency of this myopathy is unknown.

Myopathies of Exotic Animals

Although myopathies in exotic mammals are not economically significant, they are important for comparative studies with domestic animals and man.

Hamilton et al (1974) described an inherited muscular dystrophy in mink that involved progressive generalized skeletal muscle degeneration, recognizable as early as two months of age. The affected mink have an unsteady, undulating gait, and impaired running ability. Histologic changes of skeletal muscle included variation in fiber diameter size, centralization of nuclei, degeneration (including hyaline change and necrosis), increase in endomysial and perimysial connective tissue, and abortive regeneration. This myopathy has been suggested as an animal model for human muscular dystrophy.

Higginson et al (1973) reported nutritional myopathy in the Zebra. Skeletal muscle lesions were hyaline and granular, probably due to vitamin E and/or selenium deficiency. Shortly after birth, foals become stiff, then recumbent, and die in 1 or 2 days. The affected muscle appeared pale. Histopathological examination confirmed nutritional myopathy. Vitamin E and selenium supplementation prevented further losses.

Arthrogryposis has been reported in the Roe and White-tailed deer (Szabo 1965; Verme 1968; Saperstein et al, 1975b). Permanent joint contracture was found in 3 cases from Europe and 2 in Michigan. The cause of arthrogryposis in deer is unknown.

Muscle Fiber Typing and Histochemistry

Historical

As early as 1678, Stephano Lorenzini commented on different types of muscle in animals and suggested that, on the basis of color differences, they be referred to as red and white muscle (Ciacco, 1898). Ranvier (1874) after extensive studies suggested a physiological difference between red and white fibers in the rabbit, and correlated color with slow and fast contraction. His work also confirmed observations of earlier workers that red muscle had more extensive blood supply than white. Kolliker (1857) noticed the presence of interstitial granules in muscle fibers and suggested that they were probably connected with its metabolism. Retzius (1890) suggested that these granules be called sarcosomes. Grutzner (1884) tried to correlate thinner dark fibers in the gastrocnemius of the frog and the red fibers in the rabbit muscle. He expanded his work to study various other species and concluded that

muscles of all vertebrates, including man, was composed of two types of fibers--one thin and dark, the other larger and lighter in color. The dark color was attributed to granules in the sarcoplasm. However, Knoll (1891) maintained that the difference in color was due more to protoplasm between myofibrils of the darker fibers than the lighter and he divided the muscle fibers into protoplasmareiche (rich in protoplasm) and proloplasmaarme (poor in protoplasm) fibers. Knoll found that certain muscles, that were constantly active such as the ocular muscles and those of mastication and respiration, were composed mainly of dark fibers. In some animals there were muscles composed entirely of red or white muscle, while in others the fibers were mixed. For instance, in frog muscles, that appear white macroscopically, Knoll observed some small dark fibers among the large pale ones. In the higher animals only the least active muscles tended to be white. Hence the difference in the domestic fowl, where the breast muscle is white and the leg muscle red, in contrast to the birds of flight, where pectoral muscle is red and the leg muscle white. Knoll also studied the characteristics of interstitial granules and concluded they were lipoid in nature.

Lee et al (1916) studied the diaphragm, extensor longus digitorum, gastrocnemius and soleus of the cat and found

chemical and physiological differences, but they were unable to demonstrate any histological variation. However, Bullard (1919) was able to show by Sudan III staining for lipids that these muscles consisted of light, dark and intermediate fibers, and that there was a variation in proportion and size of these fibers in different muscles. A study by Denny-Brown (1929) also noted that dark fibers of cat muscle had a higher fat content than the light, but he was unable to correlate the histological features of the fibers and their speed of contraction. Using the technique of differential centrifugation for separating various structural components of muscle cells, Paul and Sperling (1952) demonstrated that white muscle had a low content of mitochondria and low respiratory activity, while red muscle was rich in mitochondria and had high respiratory enzyme activity.

Muscle Histochemistry

With the advent of modern histochemical techniques it became possible to localize enzyme systems and other chemical constituents at a cellular level and this opened the way for directly correlating functional activity of individual fibers with their morphology.

Enzymes of particular interest in studying muscle have been those connected with glycogen synthesis and breakdown

(such as phosphorylase), oxido-reductases (such as various dehydrogenases linked with the Kreb's cycle) and hydrolases (such as adenosive triphosphatase) (Dubowitz 1970).

Padykula (1952) demonstrated variation in enzyme activity of individual muscle fibers of the rat by applying and adapting the method of Seligman and Rutenburg (1951) for succinate dehydrogenase (SDH). Wachstein and Meisel (1955) also found a variation in SDH activity in fibers of various muscles of the rat and rabbit. They noted that the masseter and diaphragm contained a greater proportion of the strongly reacting fibers than did the limb muscles, while in the tongue and heart all muscle fibers reacted strongly. Similar results were obtained in various muscles of the rat by Buno and Germino (1958). At the same time, Nachimas and Padykula (1958) found that in the soleus (red muscle) the SDH reaction was uniformly strong in all fibers. On the other hand, in the biceps femoris and tibialis anterior (mixed muscles), there was a marked variation between the fibers. They observed a stronger reaction in fibers of smaller diameter and suggested that these contained more mitochondria per unit area. Investigating SDH activity in the muscles of fish, frog, bird and mammal, Ogata and Mori (1958a) recognized three fiber types--a large white fiber with weak enzyme activity, a small red fiber with strong activity and a medium fiber intermediate in size and enzyme

activity. The proportion of these three fiber types varied in different muscles. Further study including additional enzyme methods resulted in discovery of a similar variation in the cytochrome oxidase activity in the muscles of cats and mice (Ogata and Mori 1958b). George and Scaria (1958) studied the breast and leg muscles of the pigeon and fowl and were able to correlate high oxidative enzyme activity with red muscle and with high mitochondrial content.

Dubowitz (1968) began a study of the enzyme histochemistry of normal and diseased human muscle. Dubowitz and Pearse (1973) concurred with other studies that found a variation in the SDH reaction between different fibers. Further studies revealed that individual fibers reacting strongly for SDH activity were weakly reactive for all other oxidative enzymes. By the use of serial sections of animal muscles, Dubowitz and Pearse (1973) noted that fibers reacting strongly for oxidative enzymes gave a weak reaction for phosphorylase, and those reacting weakly for oxidative enzymes were strong for phosphorylase. This reciprocal activity relationship was consistently present in their study. Longitudinal sections of fibers reacted uniformly throughout its length for a given enzyme. Engel (1962) confirmed the two muscle types and the reciprocal relation of phosphorylase and oxidative enzymes in individual

enzymes in individual fibers. Engel also found that myosin ATPase was present in higher concentration in type II fibers and correlated with the higher phosphorylase content.

The researchers using histochemical muscle fiber typing have encountered difficulties in typing muscle where fibers with an enzyme activity intermediate between the strongly and weakly reacting fibers have occurred. Stein and Padykula (1962) defined three types of fibers (A, B and C) in the rat gastrocnemius on the basis of SDH reaction. They correlated the quantity of glycogen present in an individual fiber with its enzymatic activity. Their results suggested that the type A fiber represented the white fiber, and the types B and C fibers represented two types of red fibers.

Using guinea pig muscle, Edgerton and Simpson (1969) demonstrated an intermediate fiber which was slower contracting than the all red or all white fibers. These intermediate fibers were found to be type I using the myosin ATPase stain while the red and white fibers were found to be type II, or dark staining with myosin ATPase.

Muscle fiber typing was clouded by inconsistencies in the definition of fiber types and there was a lack of correlation between different systems of nomenclature. Brooke and Kaiser (1970) attempted to establish some order in muscle fiber type classification. They used the

histochemical reaction for myosin ATPase and pH lability of the reaction to characterize the various types of fibers. Type I fibers were found to correspond to those fibers that develop the greatest reaction with the oxidative enzyme stains. Type II B fibers corresponded with those fibers that develop the least reaction with an oxidative enzyme such as NADH-TR, and Type II A fibers had an intermediate staining reaction to the enzyme reaction.

Two animal scientists, Ashmore and Doerr (1971) completed a comparative histochemical study of chick, mouse, bovine and porcine muscle to determine whether their nomenclature for muscle fiber types, previously used for chick muscle, would be applicable to other species. The alpha R fibers had high ATPase activity and high SDH activity, alpha W fibers had high ATPase activity and low SDH activity, and the beta R fibers reacted weakly to ATPase and strongly for SDH. These reactions were found to be consistent for all species tested and correlated with the activity of human muscle delineated by Brooke and Kaiser (1970), but their addition to the nomenclature presented a problem to those wishing to relate their work to that of others in a muscle fiber research. The various schemes of muscle fiber nomenclature are summarized in Table 1.

Muscle biologists, Cassens and Cooper (1971) studied muscle development in the pig using histochemical myosin ATPase to determine red, white and intermediate fiber types.

A study of bovine fetal muscle fiber was conducted by Ommer (1971). He used SDH, NADH-TR and myosin ATPase to determine the presence of red, white and intermediate muscle fibers. He found that the three muscle fiber types were present at 200 days of gestation, thus related bovine muscle fiber development to observations of human muscle fiber type development.

Ariano and Armstrong (1973) did fiber type profiles of the hind limb muscles of the guinea pig, rat, cat, lesser bushbaby, and slow loris. Their results demonstrated that there was a constancy in fiber type populations within a given muscle among the five species studied.

Suzuki and Tamate (1974) found five fiber types in the muscles of steers: Type A fibers reacted strongly for myosin ATPase and phosphorylase and moderately to strongly for SDH and NADH-diaphorase; Type B fibers reacted strongly for myosin ATPase and phosphorylase and weakly for SDH and NADH-diaphorase; Type C fibers reacted weakly for myosid ATPase, weakly to moderately for phosphorylase and strongly for SDH and NADH-diaphorase; Type D fibers reacted to NADH with a reticular pattern and were larger than the C

fibers; and Type E fibers reacted very strongly for NADH and moderately for myofibrillar ATPase.

Since 1971 there has been a call in the literature for standardizing tests and fiber type classifications. Dubowitz and Brooke (1973) released Muscle Biopsy: A Modern Approach which established criterion for examining myopathies. The muscle fiber types used by Dubowitz and Brooke were three: I, IIA, IIB (Table 2). In 1977, Tunell and Hart described a simplified technique for histochemically determining all three fiber types using a single section of muscle. Their technique involved using a standard incubation time with a preincubation in a solution of formaldehyde, glycine and calcium followed by routine ATPase (Tables 3 and 4 and Appendix).

From its relatively primitive beginnings, muscle fiber typing has become a meaningful, essential tool for diagnosing and treating muscular disorders of man and animals (Manz 1980).

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II. BROWN SWISS WEAVER SYNDROME: STUDIES OF MUSCLE PATHOLOGY

INTRODUCTION

Various congenital and hereditary myopathies have been described in all animal species and man (Drachman 1961; Leipold et al, 1972; Dubowitz and Brooke 1973; Adams 1975; Saperstein et al, 1975). The causative factors may be genetic or environmental or an interaction of the two. Arthrogryposis in cattle is an example of a congenital myopathy in which both genetic and environmental agents have been described as causes (Shupe et al, 1967; Greene et al, 1973). As in the majority of congenital myopathies, arthrogryposis is clinically evident early in life. However, there is a congenital myopathy colloquially referred to as "weaver" in Brown Swiss cattle which is not clinically expressed until the animal is 6 to 8 months of age (Leipold et al, 1973).

Reported here are the histochemical fiber types and muscle pathology involved in the weaver syndrome of Brown Swiss cattle.

MATERIALS AND METHODS

Six animals were presented to the Department of Pathology from midwestern U.S.A. dairy farms and transported to Kansas State University (Table 1). All were clinically examined for signs of the weaver syndrome and humanely euthanized for complete necropsy examination. Sections of lung, liver, kidney, spleen, heart, brain, spinal cord, thyroid, adrenals and pituitary were preserved in 10 per cent neutral buffered formalin (BNF) processed and sectioned 6 microns thick, and stained with hematoxylin and eosin (H&E) and histopathological findings were noted.

The three control cows were handled similarly using the above protocol.

Muscle samples 4 x 1 x 7 cm were excised from middle of the vastus lateralis (Vl) and rectus femoris (Rf) muscles from 9 cattle. The number, age and sex of test and control cattle are given in Table 1. Muscle samples were wrapped in gauze moistened with physiological saline solution and held at 4 C for 1-12 hours. Muscle pieces 10 mm x 5 mm each were sliced with sharp blades, quenched in 2-methyl butane (isopentane) cooled to -90 C with liquid nitrogen. Serial

sections 10 um thick were cut on a cryostat[a] at -24 C, mounted on cover slips, air dried for 30 minutes at room temperature and then stained with SDH (Nachlas et al, 1958), NADH-TR (Suzuki 1977) and myofibrillar ATPase at pH 9.4 (Padykula and Herman 1955). Other sections were preincubated in 0.2 M acetic acid-sodium acid buffer at pH 4.3 and 4.6 before the ATPase reaction to determine fiber type reversal and in 0.1 M glycine calcium formal at pH 7.0 to determine 3 distinct fiber types (Tunell and Hart 1977). The remaining frozen sections were stained with H&E (Dubowitz and Brooke 1973) and modified Gomori trichrome (Engel and Cunningham 1963). Corresponding areas of the muscles were placed in 10% BNF and embedded in paraffin blocks. Serial sections of 6 um were stained with routine H&E and Gomori trichrome and phosphotungstic acid hematoxylin (PTAH).

Corresponding areas of muscle stained in serial sections for ATPase and NADH-TR were photomicrographed at a magnification of 33x on the negative. Prints were enlarged 3x to give a final magnification of 100x.

Muscle fibers were classified as type I, type IIA and type IIB and pathological changes described.

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[a]Ames Corp., Division of Miles Laboratory, Elkhart, Indiana.

RESULTS

Clinical Findings: Affected cattle walked with an abnormal gait and had problems placing their feet correctly as they moved. This resulted in weaving, dog-tracking or walking somewhat sideways. Tendon reflexes were normal. The cattle tend to stand with their front legs spread out wider than their shoulders while their hind legs are placed almost together. Two animals (619, 1126) lost rear limb control and were recumbent for 1 week (619) and 2 days (1126) prior to euthanasia. Histories revealed clinical signs were detected from 6 to 8 months of age. Pedigrees of affected cattle reveal common ancestors, but the mode of inheritance was not ascertained.

Gross Findings: All affected cattle displayed a marked, generalized atrophy of the muscles of the hind quarters, yet gross lesions were limited to the M. vastus lateralis (Figures 1-6). The two recumbent animals (619, 1126) had some subcutaneous bruising and edema of the fascicular planes. The skin was scraped in the area of the tarsus and carpus. All joints were examined and found to be normal as well as by bi-sections of the femur and humerus. The heart, lungs, liver, spleen, kidneys and adrenals were normal, with the exception of 619 that had some pneumonia. Examination of brain, spinal cord and nerves of the hind quarter revealed

no gross changes. Lesions of the M. vastus lateralis varied from slight (1083, 1372, 1523), to moderate (1304), to severe (619, 1126). The lesion consisted of a peripheral whorling of fat and connective tissue from 3 to 6 cm, often without any muscle tissue present. The affected muscle was pale in comparison to the rest of the quadriceps group. The muscle was smaller than normal and was more difficult to cut.

Control cattle revealed no gross lesions.

Microscopic Findings: Sections of affected muscle displayed a whorling of amorphous substance with small, triangular fibers present. Other sections had many nuclei and a considerable interfibrillar substance. There are fascicles in which there were smaller, larger and intermediate fibers (Figures 7-12). A common feature of affected muscle sections was replacement of muscle fibers by fat and lack of regeneration (Figures 8-12). Modified Gomori trichrome stain was used to assess the amount of collagen present in a section. The various structures in the muscle sections differentiated by staining are summarized in Table 3.

Histochemical stains revealed how the fiber types reacted to the disease. The myofibrillar ATPase reaction at pH 9.4 differentiated two fiber types, type I and type II, but preincubation with Tunell's glycine buffered calcium formal (GBCF) revealed three fiber types (type I, type IIA

and type IIB) of the affected muscle (Figures 13-24). Confirmation of the ATPase stains by NADH-TR method was successful. Type I fibers stained densely with diffuse granules, type IIA fibers with coarse granules concentrated beneath the sarcolemma and type IIB stained sparsely with large granules (Figures 19-24). Histochemical stains revealed the small fibers as type I, the large fibers as type IIB, and the normal size fibers as type IIA. Sections of M. rectus femoris were normal for fiber type and size. Staining results of serial sections using NADH-TR and SDH were similar except that tetrazolium deposits were heavier with the NADH-TR (Figures 16 and 20).

The GBCF preincubation technique (Figure 17) was more reliable than acid preincubation using acetate buffers.

DISCUSSION

The control animals provided information necessary for comparing muscle fibers and their reaction to disease. The control muscle was easy to fiber type in contrast to affected weaver muscle which met with 70% success. This resulted from excess of collagen, lipid replacement of muscle fibers, and freezing difficulties. The GBCF pre-incubation and NADH-TR provided the best resolution of the three fiber types. These techniques have also been proven to be successful for analyzing human muscle fiber types (Dubowitz and Brooke 1973; Manz 1980).

The small, dark fibers were found to be type I, the intermediate size and staining fibers were type IIA, and the large, light staining fibers were type IIB in character. These sections were examples of hypertrophy of the type IIB fiber along with atrophy of type I fibers. The relative excessive number of nuclei is due to the smaller atrophied fibers. Although fibroplasia is a common response to atrophy, it was not evident in affected muscle sections.

Other reports indicated no difficulty in obtaining reliable results with the myosin ATPase reaction in the rat (Padykula and Herman 1955) dog (Cardinet 1971), horse (Lindholm and Piehl 1974), mouse, guinea pig, cat (Dubowitz 1968) and sheep (White et al, 1978). Success in fiber

typing of affected Brown Swiss was increased by collecting the muscle immediately after euthanasia and holding it in physiologic saline moistened gauze for no more than 4 hours. Dubowitz and Brooke (1973) suggested that fresh $(NH_4)_2S$ for precipitating the cobalt ion in the ATPase staining was essential for obtaining consistent results.

The acid preincubation of bovine muscle did not always delineate the three fiber types as reported by Dubowitz and Brooke (1973) in human muscle and in rat muscle (Samaha et al, 1970). The difficulty of differentiating type IIA and type IIB fibers by this technique made reliable classification impossible. Since the M. vastus lateralis and M. rectus femoris are of mixed fiber types, the most reliable information was obtained by use of the GBCF and NADH-TR techniques.

Ommer (1971) reported muscles differentiated into 2 fiber types by day 260 of gestation. Studies by Ashmore et al (1972) concurred and added that fiber typing became more reliable as age increased. Since the cattle in this study were all at least 1 year old, the difficulty in fiber typing is attributed to artefact and pathological changes. Since all suspect cattle had similar clinical signs, peculiar gait, marked atrophy of hind quarters, and parentage, a genetic-biochemical defect may be postulated.

Muscle is limited in its pathological changes. Denervation will cause a decrease in diameter of all fiber types in a muscle fascicle, but spinal problems with loss of specific motor neurons cause atrophy in one type of fiber (Dubowitz and Brooke 1973). Starvation causes atrophy or lessening of all fiber type diameters (Swatland 1978). Disuse atrophy decreases the diameter of type II fibers and training causes hypertrophy (Dubowitz and Brooke 1973) and a proportional increase in type IIA fibers (Lindholm and Piehl 1974). Muscular dystrophies produce decreases in particular fiber type diameters and cause a change in proportion resulting in a fiber type deficiency or predominance (Dubowitz and Brooke 1973).

Congenital myopathies are a new generation of genetically determined muscle disorders with clinical signs similar to muscular dystrophies or neurogenic atrophies, but in which specific structural abnormalities can be recognized in the muscle (Dubowitz 1968). Adams (1975) defined muscular dystrophy as a genetically determined, progressive, nonregenerative myopathy. Weaver syndrome has been classified as a muscular dystrophy by Leipold et al (1973). The problem is to determine the primary muscular abnormality in weaver syndrome. Could it be that the initial change is hypertrophy of type IIB fibers, followed by atrophy of type I fibers, or is the hypertrophy of IIB fibers a result?

Ashmore and Doerr (1972) reported that type IIA fibers are capable of becoming type IIB fibers after birth. If this is true, then there should be an excess number of group IIB fibers with a corresponding decrease in IIA fibers. This is not the case, however, since there is no evidence of one type predominating affected muscles. Proliferation of collagen and replacement of muscle fibers by lipid are, in this case, secondary to fiber atrophy. The primary lesion or feature is atrophy of type I fibers which are essential for maintaining posture. Type I fibers have many small mitochondria and are oxidative in metabolic characteristics making them susceptible to biochemical disorders. Until the biochemical defect can be identified, the weaver syndrome remains as a muscular dystrophy similar to the Duchenne type of humans.

SUMMARY

The muscle pathology and histochemical fiber types of Brown Swiss weaver syndrome was studied in six affected and three control cattle. Muscle fibers were classified by myosin adenosine triphosphatase and nicotine amide adenosine dinucleotide-tetrazolium reductase methods for determining fiber types (I, IIA, IIB).

The abnormal, weaving gait was present at 6 to 8 months of age. A peripheral, whorling, 3 to 6 cm lesion of fat and connective tissue was found in the M. vastus lateralis of affected cattle. Microscopic features were atrophy and hypertrophy of muscle fibers without regeneration, replacement of muscle fibers by fat and many nuclei with an excess of interfibrillar collagen. Muscle fiber typing determined the atrophic fibers to be type I, the hypertrophic fibers type IIB and intermediate fibers type IIA.

The primary lesion type I fiber atrophy associated with nonregenerative fiber replacement by fat. This classified the weaver syndrome as a muscular dystrophy.

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TABLE 1--Cattle studied for characterizing muscular pathology of the weaver condition in Brown Swiss.

Number	Age	Sex	Weight (kg)
619	2 yrs.	Female	500
1083	18 mos.	Male	545
1126	2 yrs.	Female	454
1304	2 yrs.	Male	420
1372	1 yr.	Female	364
1523	2	Female	523
Control 1 (Jersey)	2 yrs.	Female	400
Control 2 (Jersey)	18 mos.	Female	364
Control 3 (Holstein)	2 yrs.	Female	522

TABLE 2--Comparative mammalian muscle fiber type nomenclature.

Author(s)	Species	Fiber Types		
Dubowitz and Brooke (1973)	human	I	IIA	IIB
Brooke & Kaiser (1970)	human	I	IIA	IIB
Ogata & Mori (1958)	mammals	medium	red	white
Stein & Padykula (1962)	rat	B	C	A
Engel (1962)	human	I	II	II
Romanul (1964)	rat	III	II	I
Padykula & Gauthier (1967)	rat	intermediate	red	white
Yellin & Guth (1970)	rat, rabbit	beta	alpha beta	alpha
Ashmore & Doerr (1971)	cattle, swine	beta red	alpha red	alpha white
Barnard et al (1971)	mammals	slow twitch intermediate	fast twitch red	fast twitch white
Peter (1972)	guinea pig, rabbit	slow twitch oxidative	fast twitch oxidative-glycolytic	fast twitch glycolytic
Khan et al (1973)	mammals	I red	II red	II white
Lindholm & Piehl (1974)	horse	slow twitch	fast twitch high oxidative	fast twitch
Suzuki (1977)	cattle, sheep	C	A	B

TABLE 3--Characteristics of mammalian muscle fiber types.

General Characteristics	Muscle Fiber Types		
	Type I	Type IIA	Type IIB
Natural color	dark	dark	pale
Surrounding capillary density	high	high	low
Relative histochemical activity			
Myosin ATPase pH 9.4	low	high	high
Myosin ATPase pH 4.6	high	low	high
Myosin ATPase pH 4.3	high	low	low
Succinic dehydrogenase	high	high	low
NADH-TR	high	intermediate	low
Glycogen	low	high	high
Myoglobin	high	high	low
Physiology			
Twitch speed	slow	fast	fast
Fatiguability	resistant	resistant	sensitive
Dynamic use	postural activity	phasic, volitional contraction	fast phasic volitional contraction
Electron microscopic features			
Mitochondria	many, small	many, large	few, small
Z disc	intermediate	wider	narrower

TABLE 4--Site of histochemical reactions of mammalian skeletal muscle cells.

Reaction	Normal Localization		Source of Reaction	Specificity	Technique
	Transverse	Longi-tudinal			
Myofibrillar ATPase (acid or alkali pH)	Myofibrillar	A band	Myosin or actomyosin	Good	Brooke and Kaiser (1970)
NADH-TR	Intermyofibrillar	I band	Mitochondria, sarcoplasmic reticulum & tubules	Fair	Brooke and Engel (1966a)
Succinic dehydrogenase	Intermyofibrillar	I band	Mitochondria	Excellent	Ogata and Mori (1958a)

TABLE 5 -- Differentiating staining features of mammalian muscle sections.

Structures	Hematoxylin and Eosin	Modified Trichrome Engel, Cunningham (1963)
Muscle fibers		
myofibrillar space	pink	green (Z disc is red)
intermyofibrillar space	blue	red
nuclear chromatin	blue	purple
Connective tissue	pink	light green
Myelin of nerves	blue	red
Axons	purple	purple
Spindle capsule	pink	green
Blood vessels	pink	green

THIS BOOK CONTAINS NUMEROUS PAGES THAT WERE BOUND WITHOUT PAGE NUMBERS.

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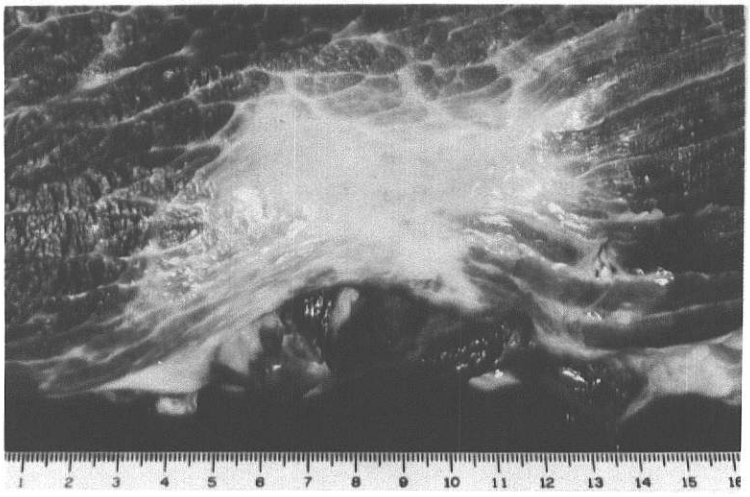
Figure 1: Muscular changes in Brown Swiss weaver syndrome. Cross section of vastus lateralis muscle. Notice peripheral whorling of fat and collagen. The scale is in centimeters, necropsy number 79-1126.

Figure 2: Brown Swiss weaver syndrome. Cross section of vastus lateralis muscle of necropsy number 79-1523. Notice fat and collagen replacing muscle fibers. The scale is in centimeters.

Figure 3: Longitudinal, clamped, vastus lateralis muscle sample from Brown Swiss cow (79-619) affected with weaver condition. Notice paleness of muscle.

Figure 1



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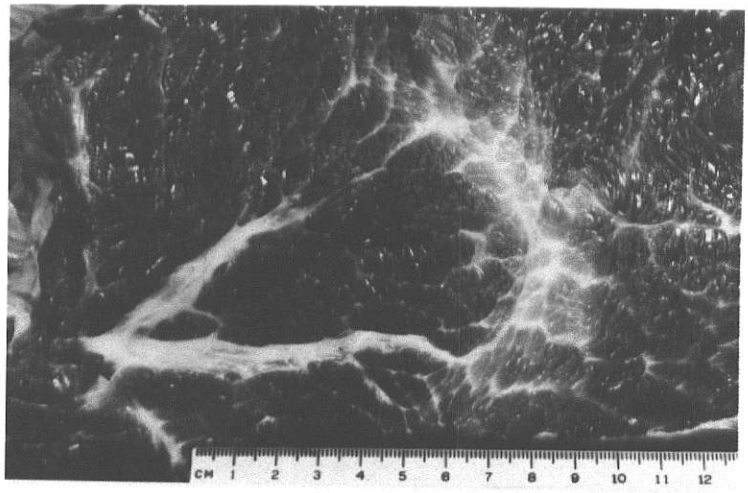

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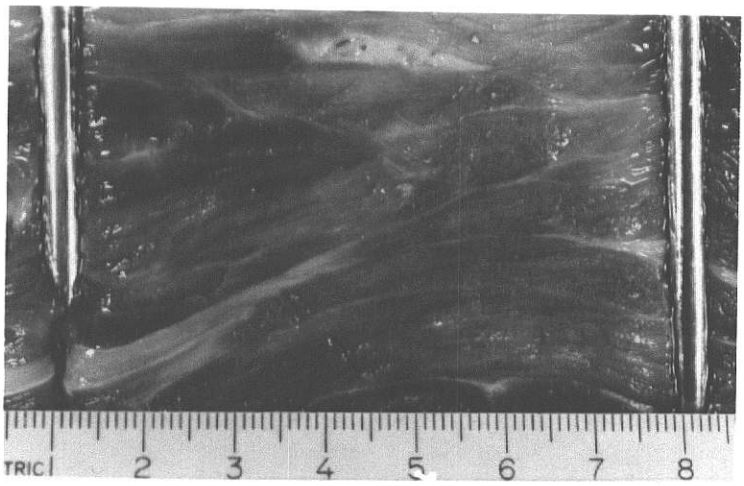
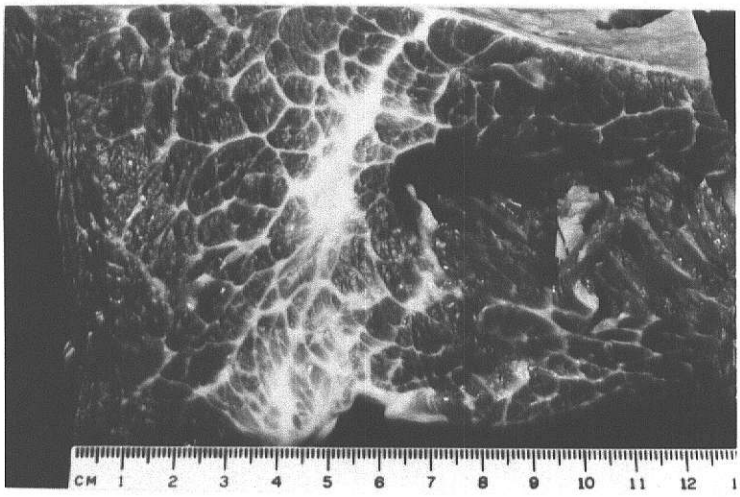


Figure 4: Gross muscular changes encountered in Brown Swiss weaver syndrome. Cross section of vastus lateralis. Note the 2 cm band of fat and connective tissue bisecting the muscle. The whorling of connective tissue is present in the center of this lesion. Necropsy number 79-1304.

Figure 5: Cross section of vastus lateralis muscle of necropsy number 79-1372. This slightly affected Brown Swiss heifer revealed a pale, triangular area in the center of the muscle.

Figure 6: Clamped, cross section of vastus lateralis muscle from Brown Swiss cow affected with weaver syndrome, necropsy number 79-1126. Notice the pale, edematous nature of this muscle.

Figure 4



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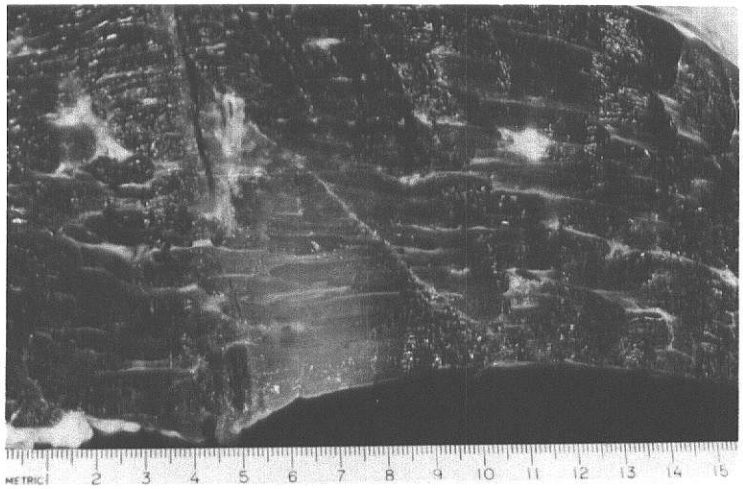

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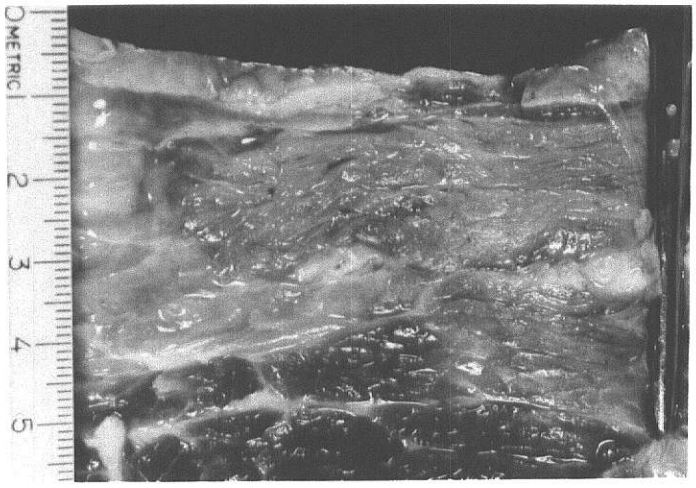
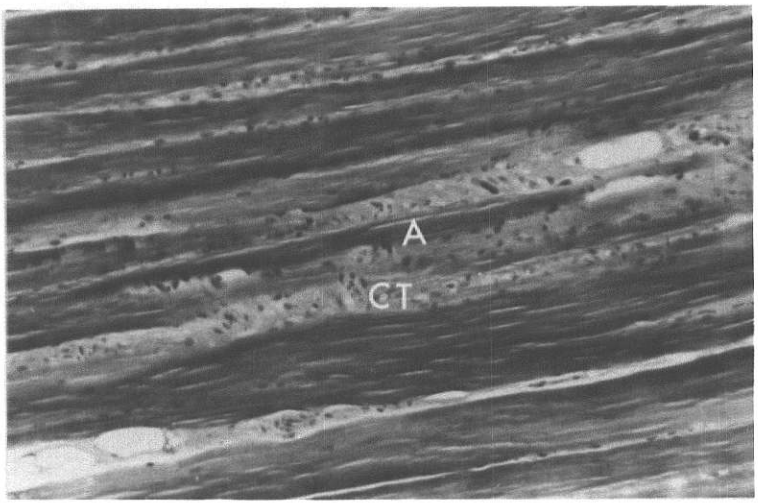


Figure 7: Photomicrograph of sample of muscle tissue from vastus lateralis muscle of a Brown Swiss cow (79-1126) affected with weaver syndrome. Notice atrophy of muscle fibers (A) accompanied by an increase in connective tissue (CT). Trichrome stain 10X.

Figure 8: Photomicrograph of cross section of affected vastus lateralis muscle. Notice atrophy of muscle fibers (A) and their widespread replacement by connective tissue (CT). Trichrome stain 10X.

Figure 9: Photomicrograph of longitudinal section of vastus lateralis muscle from a Brown Swiss bull (79-1083) affected with weaver syndrome. Notice the replacement of degenerated muscle fibers by lipid material (arrow). H&E 10X.

Figure 7



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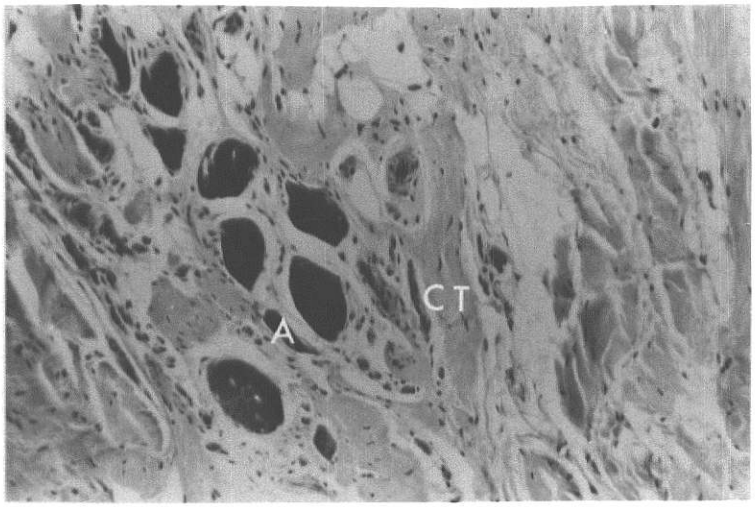

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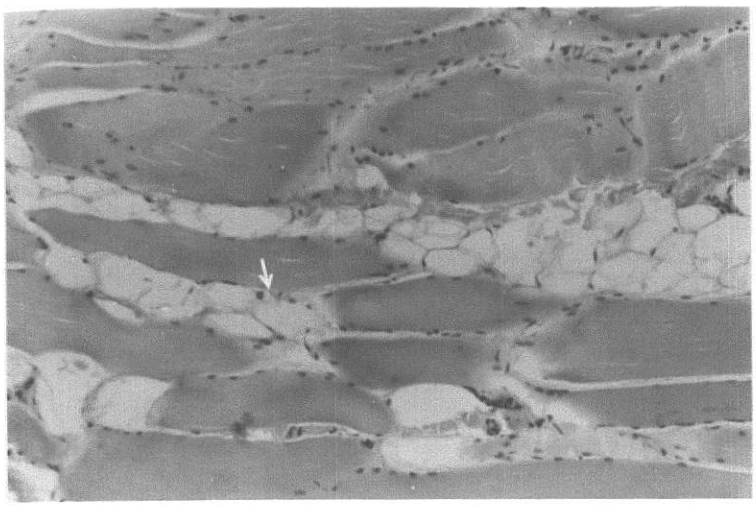
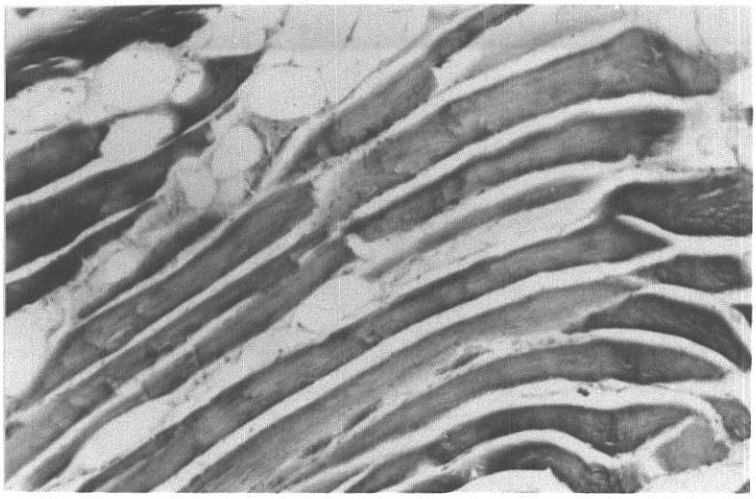


Figure 10: Photomicrograph of a sample of muscle tissue from vastus lateralis muscle of a Brown Swiss cow (79-1523) affected with weaver syndrome. Notice atrophy of muscle fibers. Phosphotungstic acid haematoxylin (PTAH) stain 10X.

Figure 11: Photomicrograph of a longitudinal section of vastus lateralis muscle of a Brown Swiss cow (79-1126) affected with weaver syndrome. Notice atrophy of muscle fibers (A) accompanied by lipid replacement (L). PTAH stain 10X.

Figure 12: Photomicrograph of longitudinal section of vastus lateralis muscle of a Brown Swiss cow (79-1523) affected with weaver syndrome. Note the presence of degeneration of muscle fibers without evidence of regeneration (D). The arrow indicates lipid replacement of degenerating muscle fibers. PTAH stain 10X.

Figure 10



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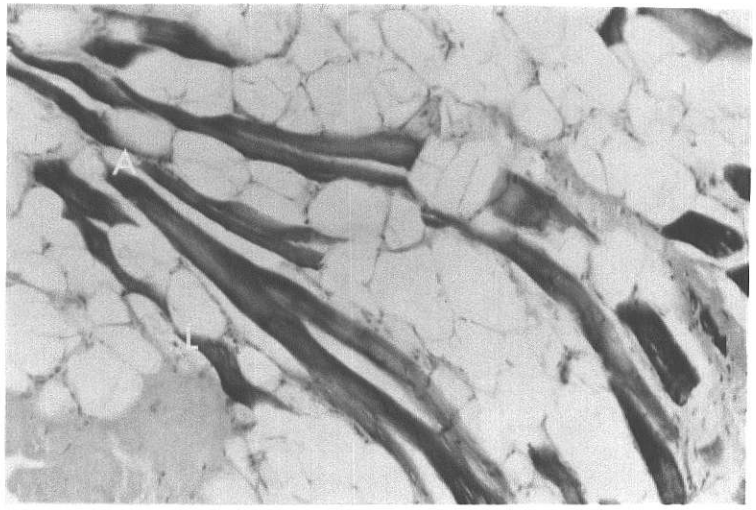

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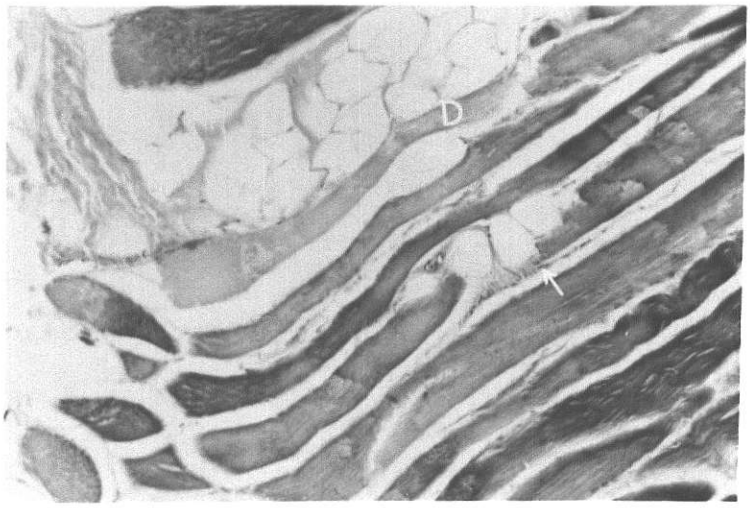


Figure 13: Photomicrograph of cross section of vastus lateralis from normal control animal. Frozen section was preincubated for 5 minutes at pH 4.6. Muscle fibers of types I and IIB (I) stained dark, while type IIA fibers (II) stained light. Notice uniform, slight size variation of muscle fibers. ATPase stain pH 4.6. 10X.

Figure 14: Photomicrograph of vastus lateralis of Brown Swiss bull (79-1304) affected with weaver syndrome and treated as described above. Notice variation of fiber size. Type I fibers (I) are small and triangular while type IIA fibers (II) are uniform and regular in shape. ATPase stain pH 4.6. 10X.

Figure 15: Photomicrograph of vastus lateralis muscle of a Brown Swiss bull (79-1304) affected with weaver condition. Frozen section was preincubated at pH 5.7 for 5 minutes prior to routine ATPase staining. Type I muscle fibers stained light (I) while type II fibers (II) stained dark. Note staining reversal when compared to Figures 13 and 14. ATPase stain pH 5.7. 10X.

Figure 13

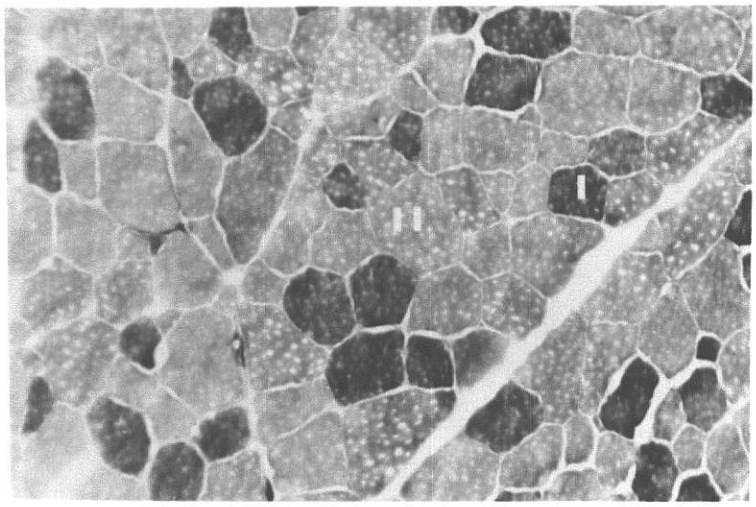
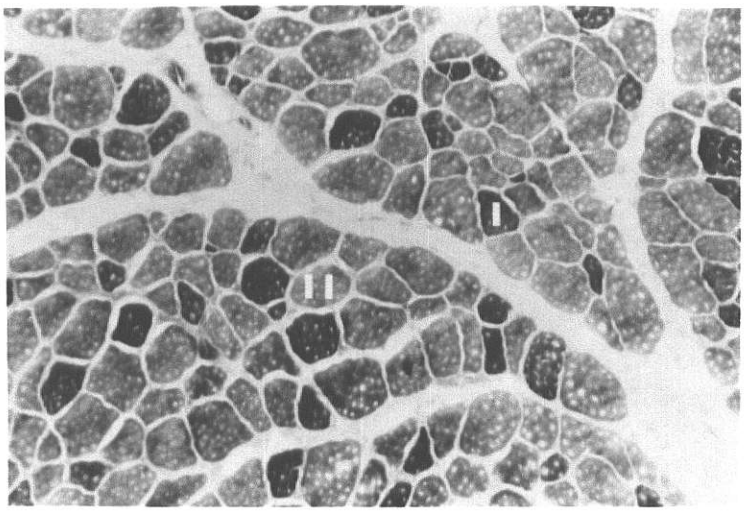


Figure 14



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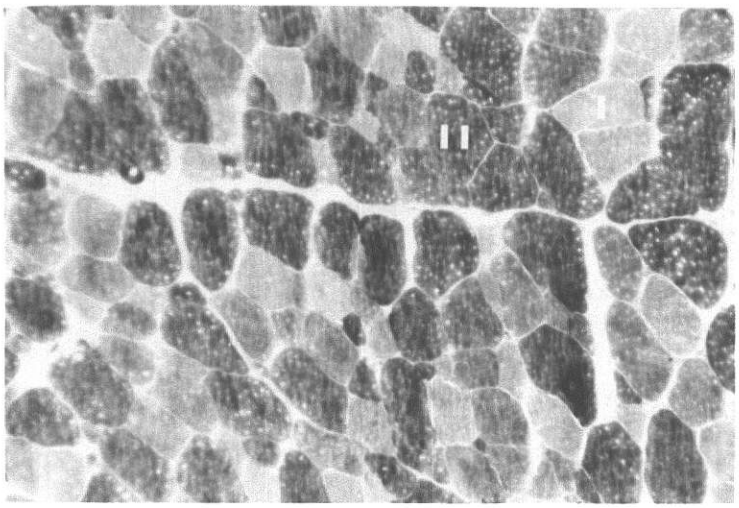


Figure 16: Photomicrograph of frozen muscle sample, cross section of vastus lateralis, case number 79-1523. Succinic dehydrogenase stain was used to differentiate two fiber types. Type I and IIA fibers stained dark (I). Type IIB fibers stained light (II). Note the size variation within the dark staining fibers. SDH stain 10X.

Figure 17: Photomicrograph of frozen muscle sample, cross section of vastus lateralis, case number 79-1126. ATPase stain preincubated with glycine calcium-formalin at pH 7.0 for 5 minutes. Three fiber types are differentiated. Type I fibers are dark (I), type IIA fibers stain intermediate (IIA) and type IIB fibers stain light (IIB). Notice the large type IIB fibers and the irregular shape of the type I fibers. ATPase stain pH 7. 10X.

Figure 18: Photomicrograph of frozen cross section of vastus lateralis muscle of control animal. Routine ATPase reaction at pH 9.4 was able to differentiate two fiber types. Dark staining type I fibers (I) and light staining type II fibers (II). Note uniform fiber size. ATPase stain pH 9.4. 10X.

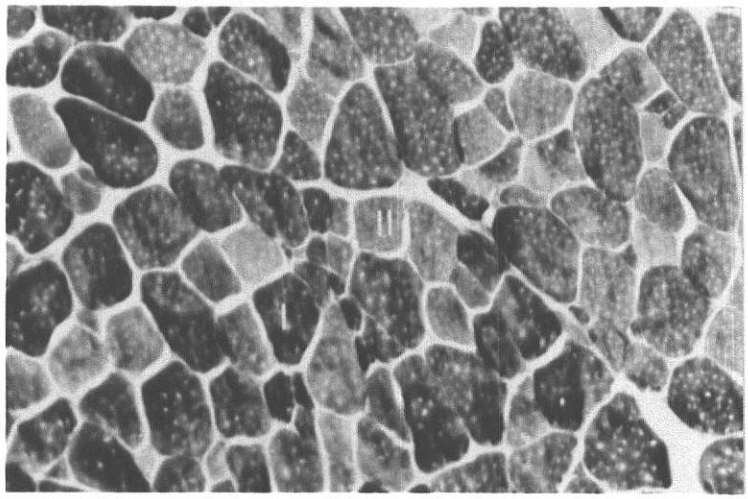


Figure 16

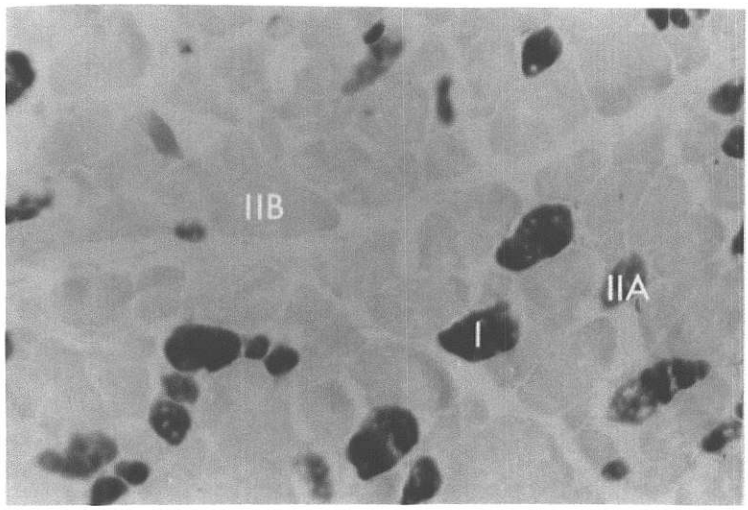


Figure 17

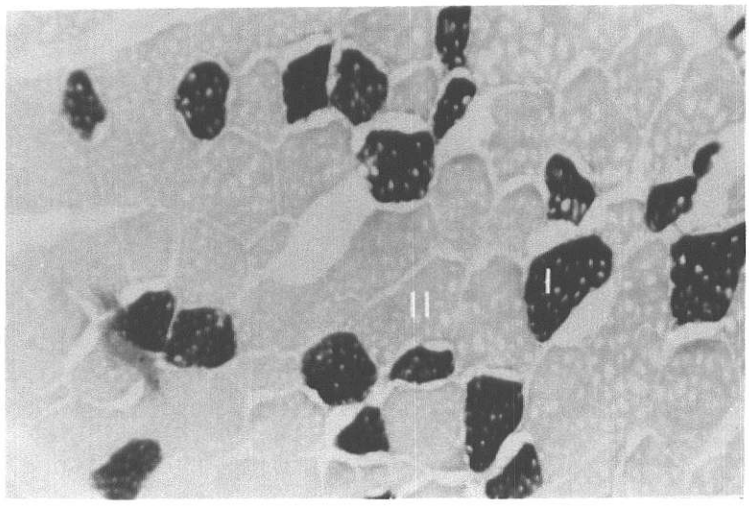


Figure 18

These photomicrographs of frozen muscle sections of vastus lateralis muscle prepared using NADH-TR stain to differentiate muscle fiber types. Type I fibers stain dark (I), type IIA fibers stain intermediate (IIA) and type IIB fibers stain light (IIB).

Figure 19: Photomicrograph of control animal. Note the uniformity of fiber type groups. NADH-TR. 10X.

Figure 20: Photomicrograph of case number 79-1304. Note extreme fiber size variation. Type I fibers are small and triangular in shape (I) in contrast to large type IIB fibers (IIB). NADH-TR. 10X.

Figure 21: Photomicrograph of case number 79-1083. Note that the type IIB fibers are not as numerous (IIB) as in control section (Figure 19). Type I fibers are atrophied and triangular (I). NADH-TR. 10X.

Figure 19

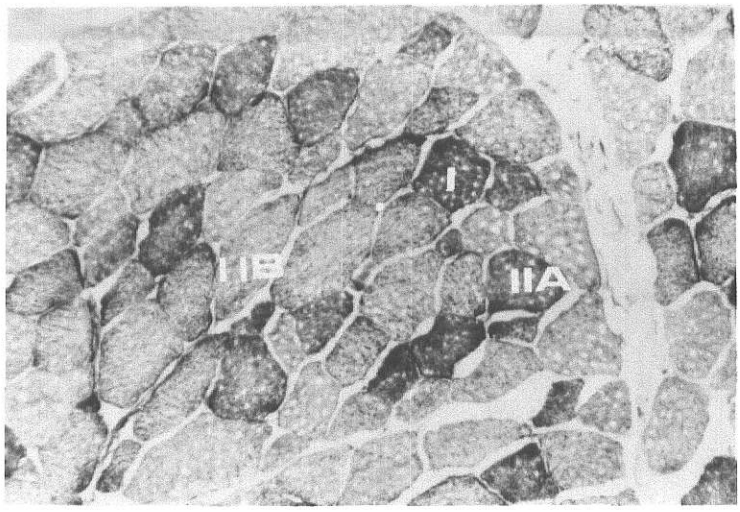


Figure 20

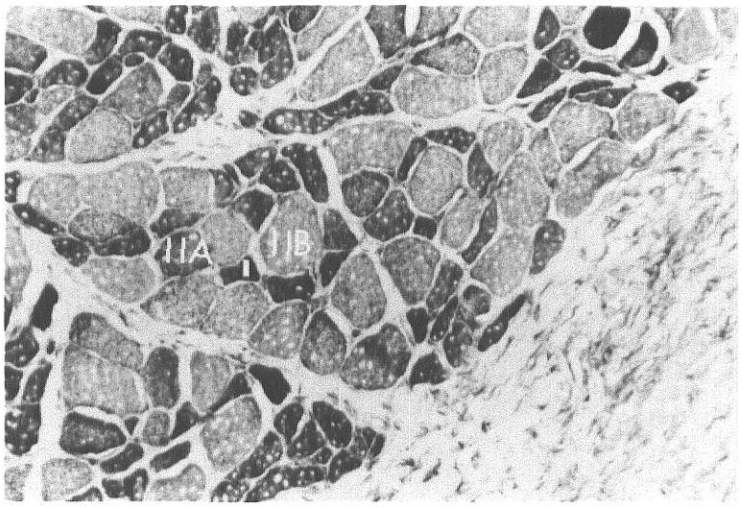
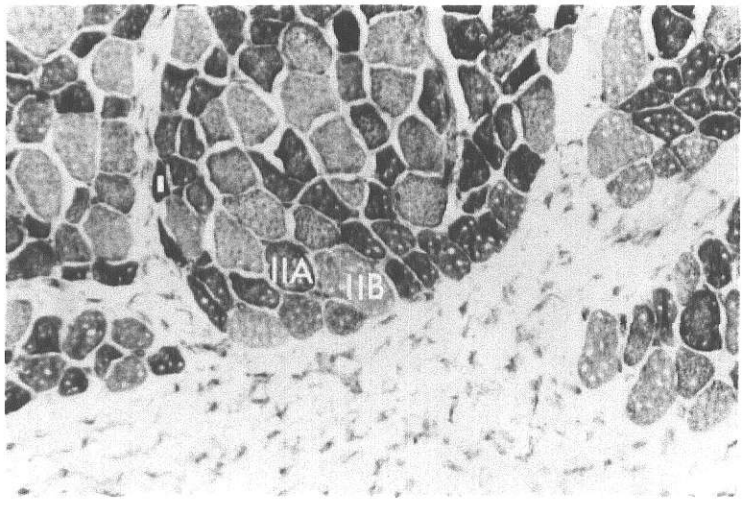


Figure 21



These photomicrographs of frozen muscle samples of vastus lateralis from Brown Swiss cattle affected with weaver condition. NADH-TR stain was used to differentiate muscle fiber types. Type I fibers stained dark (I), type IIA fibers stained intermediate (IIA), type IIB fibers stained light (IIB).

Figure 22: Affected heifer 79-1372. Type I fibers are atrophied and triangular in shape (I). Type IIB fibers (IIB) are larger while type IIA fibers (IIA) are normal in appearance. NADH-TR. 10X.

Figure 23: Affected cow 79-619. Notice the isolation of type I fibers by connective tissue (I) and grouping of type IIB fibers (IIB). NADH-TR. 10X.

Figure 24: Affected cow 79-1126. Notice the collagen in lower portion of photomicrograph which adjoins the atrophied type I fibers (I). The type IIA fibers (IIA) are normal in size in contrast to the hypertrophied type IIB fibers (IIB). NADH-TR. 10X.

Figure 22

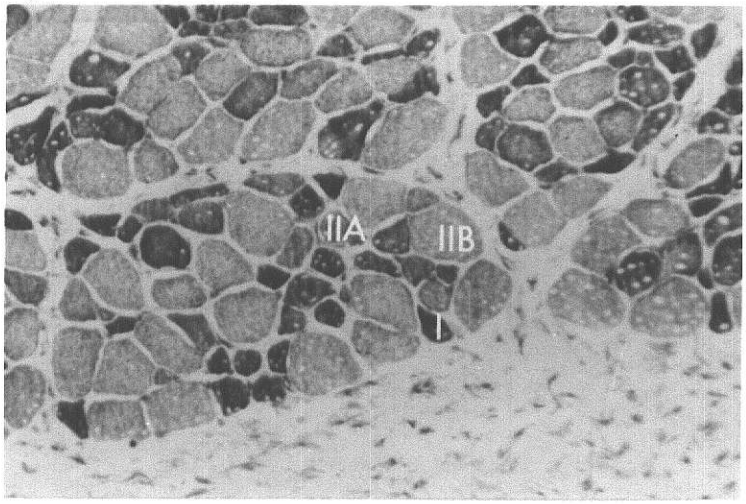


Figure 23

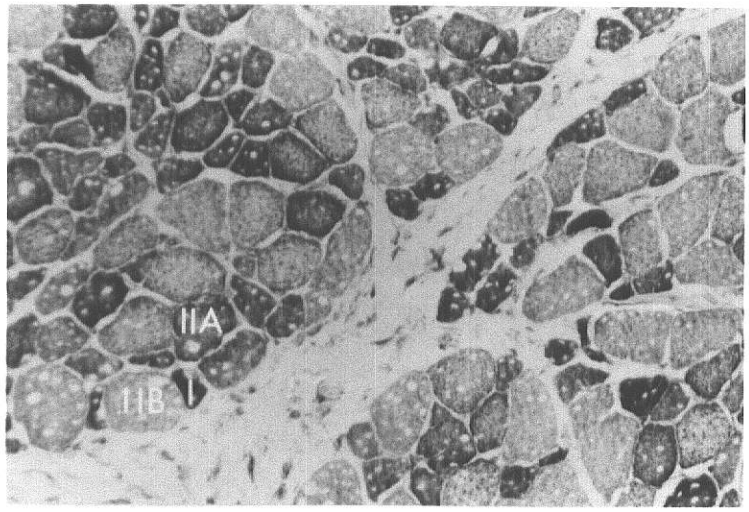
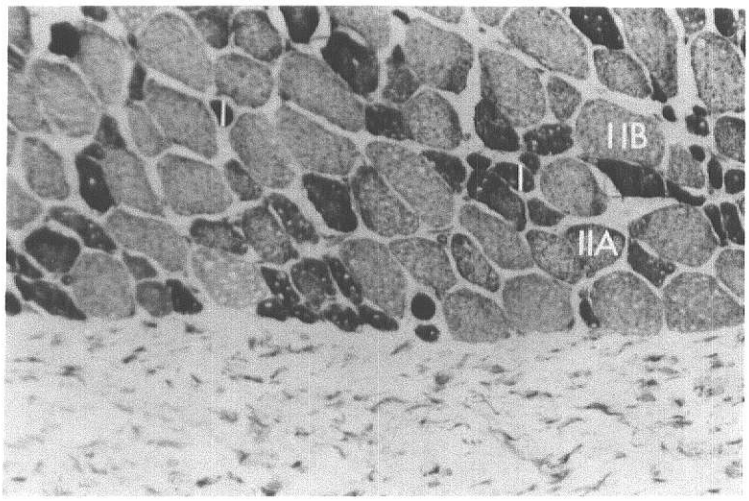


Figure 24



APPENDIX

HISTOCHEMICAL TECHNIQUES

1. Muscle Sample Collection

--Samples were collected from recently euthanatized cattle.

--M. vastus lateralis was sampled from the middle of the muscle, 4 x 1 x 7 cm section with the long edge parallel to the muscle fibers.

--M. rectus femoris was sampled in the middle of the muscle where a 4 x 1 x 7 cm section was removed with the long edge running parallel to the fibers.

--Samples were placed in gauze moistened with physiologic saline and stored for 1 to 5 hours at 4 C.

2. Quenching

--While still cold from refrigeration the muscle sample was cut in transverse sections less than 5 mm thick.

--Sections 5 mm x 5 mm were placed on 1 cm squares of thick, brown paper using mounting medium.

--2-methyl butane in a glass beaker was lowered into liquid nitrogen in a Dewar flask.

--The 2-methyl butane was allowed to cool to -80 C or to the point at which there was crystal formation at the bottom of the beaker.

--The muscle, attached to the paper squares was quenched in the 2-methyl butane for 5 to 10 seconds and removed when convection currents at the surface of the muscle stopped.

--The frozen muscle sample was placed in a vial precooled at -30 C and sealed with a tight fitting cap.

--The labeled vial was stored at -60 C.

3. Frozen Sectioning of Muscle

--Muscle in vials was removed from storage and placed in the cryostat cabinet at -26 C.

--Muscle samples were attached to microtome chucks using mounting medium.

--The chuck was placed on the microtome and the face of the muscle was leveled off.

--10 um sections were collected serially and picked up on warm (22 C) cover slips.

--The cover slips were placed in labeled Columbia jars and allowed to air dry for 60 minutes.

--The sections were either stained immediately or stored in tightly capped jars at -30 C for up to 10 days before staining.

--At the end of storage, Columbia jars with cover slips were allowed to warm to room temperature before removing the cap to prevent condensation on the cover slips.

4. Adenosine Triphosphate Hydrolase (ATPase) with Tunell's pH 7 Preincubation

--Air dry frozen 10 um sections 60 minutes.

--Preincubation air dried sections for 5 minutes in 0.1 M glycine, 1% calcium chloride, 10% formaldehyde solution adjusted to pH of 7.25.

--Rinse with distilled water 5 times.

--Add 15.2 mg. adenosine triphosphate (#A5394 Sigma):

2 ml. 0.1 M barbital

1 ml. 2% calcium chloride

7 ml. distilled H_2O

Mix and adjust to pH 9.4 with 1 N NaOH.

--Incubate sections in this medium at 37 degrees C for 2 hours.

--Wash with 1% $CaCl_2$ solution 3 to 4 times.

--2% $CaCl_2$ solution for 3-5 minutes.

--Wash thoroughly 10 times over 5 minutes in distilled water.

--Place cover slips in 1% $(NH_4)_2S$ solution for 30 seconds.

--Wash out $(NH_4)S$ solution with tap water thoroughly and hold in water.

--Dehydrate in 95% and 100% alcohol.

--Clear in xylene and mount in synthetic medium.

5. ATPase with Acid Preincubation at pH 4.6, 4.45 and 4.3

--Air dry 10 um frozen sections for 60 minutes.

--Preincubate for 5 minutes in 0.1 M Na acetate buffers adjusted to pH 4.6, 4.45 or 4.3 using 1 N NaOH or 1 N acetic acid.

--Wash five times in distilled water.

--Place in ATPase incubating medium at pH 9.4 for 30 minutes.

--Follow procedure for normal ATPase development.

6. Nicotinamide Adenine Dinucleotide-Tetrazolium Reductase (NADH-TR) (Suzuki, 1977)

--Air dry 10 um frozen sections for 60 minutes.

--Prepare 20 ml. of incubating medium from:

10 ml. of 0.2 M Tris buffer at pH 7.2

10 ml. of distilled water

10 mg. of Nitro-BT

5 mg. of NADH (reduced)

--Place cover slips in incubating medium for 90 minutes to develop blue stain.

--Place cover slips in 10% BNF for 10 minutes.

--Wash and hold in distilled water.

--Dehydrate in 95% and 100% alcohol.

--Clear in xylene and mount in synthetic medium.

7. Succinic Dehydrogenase (SDH) (Nachlas et al, 1957)

--Air dry 10 um frozen sections for 60 minutes.

--Prepare substrate from equal volumes of:

0.2 M sodium succinate

0.2 M phosphate buffer adjusted to 7.6 pH

--Mix:

10 ml. substrate

10 ml. distilled water

10 mg. Nitro-BT

--Incubate cover slips for 1 hour at 30 C in medium.

--Wash with physiologic saline solution.

--Dehydrate in 95% and 100% alcohol.

--Clear in xylene and mount in synthetic medium.

8. Modified Gomori Trichrome (Engel, Cunningham, 1963)

--Air dry 10 um frozen sections for 60 minutes.

--Harris hematoxylin for 5 minutes (filter first).

--Rinse in tap water.

--Stain in Gomori for 3-10 minutes.

--Dip in 0.2% acetic acid.

--Dehydrate in graded alcohols, clear in xylene and mount with synthetic medium.

Formulas for Solutions Used in Histochemistry

--0.1 M barbital, 20.62 g of Na barbital in one liter of distilled water. Store in refrigerator.

--2% calcium chloride solution, 20 g $CaCl_2$ in one liter of distilled water. Store in refrigerator.

--1% calcium chloride solution, 10 g $CaCl_2$ in one liter distilled water.

--2% cobalt chloride, 20 g $CaCl_2$ in one liter of distilled water.

--0.1 M glycine-calcium chloride formal (Tunell's preincubation):

Dissolve 7.51 g glycine and 10 g $CaCl_2$ in distilled water to make 900 ml. Add 100 ml. 100% formalin and store at 4 C. pH 7.25.

--1% (dilute) $(NH_4)_2S$ solution 2 ml. of 20% $(NH_4)_2S$ in 100 ml. of distilled water. Make fresh before each use.

--0.2 M acetate buffer:

Solution A--Add 1.2 ml. glacial acetic acid to 100 ml. of distilled water.

Solution B--Na acetate 2.7 grams q.s. to 100 ml. with distilled water. Add solution A to solution B using pH meter to arrive at pH 4.3, 4.45 or 4.6.

--0.1 M acetate buffer:

Solution A--Add 0.6 ml. glacial acetic acid to 100 ml. of distilled water.

Solution B--Na acetate 1.35 g q.s. to 100 ml. with distilled water.

Add solution A to solution B using pH meter to arrive at pH of 4.3, 4.45, 4.6 or 5.7.

--0.2 M Tris buffer:

Add 12.1 g Tris base and 1.863 ml. conc HCL to 400 ml. of distilled water q.s. to 500 ml. with distilled water. Adjust to pH 7.2 with 1 N NAOH or 1 N HCL as needed.

--0.2 M sodium succinate:

Add 32.4 g Na succinate to one liter of distilled water.

--0.2 M sodium phosphate buffer: 0.3312 g of NaH_2PO_4 and 4.7168 g. NaH_2PO_4 to 100 ml. of distilled water x pH 7.6.

--Gomori stain:

0.6 g chromotrope 2R or Panceau 2R

0.3 g fast green FCF or light green SF yellowish

0.6 g phosphotungstic acid

1.0 ml glacial acetic acid

Distilled water to 100 ml.

Adjust pH to 3.4 with 1N NaOH

NOTE: Solution is good for 7 to 10 days. Purity of chemicals is critical, take extra care not to contaminate them while preparing this stain.

BROWN SWISS WEAVER SYNDROME: STUDIES OF MUSCLE PATHOLOGY

by

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B.S., Kansas State University, 1978

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

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Manhattan, Kansas

1980

The histochemical fiber types and myopathy of the Brown Swiss weaver syndrome was studied. Six affected and three control cattle were subjected to clinical, gross and histopathological examinations. Fiber type and size of quenched quadriceps muscles were classified using myosin adenosine triphosphatase and nicotinamide adenosine dinucleotide-tetrazolium reductase stained sections. The M. vastus lateralis and M. rectus femoris were mixed muscles containing three fiber types (I, IIA, IIB). Paraffin sections of quadriceps muscles were stained with haematoxylin and eosin, modified Gomori trichrome and phosphotungstic acid hematoxylin for histopathological evaluation.

Affected cattle walked with an abnormal gait, resulting in weaving, dog-tracking or walking somewhat sideways. Clinical signs became apparent at 6 to 8 months of age and eventually resulted in recumbency. Pedigrees revealed common ancestors but the mode of inheritance was not determined.

A marked, generalized atrophy of the quadriceps muscles with lesions confined to the M. vastus lateralis was found in affected Brown Swiss cattle postmortem. Lesions, 3 to 6 cm in diameter, consisted of peripheral whorling of fat and connective tissue, often without any muscle tissue evident.

Microscopic features were atrophy and hypertrophy of muscle fibers without regeneration. Replacement of muscle fibers by fat as well as many muscle nuclei and large amount of collagen were commonly found. Histochemical fiber typing of M. vastus lateralis revealed small fibers as type I, large fibers as type IIB and normal size fibers as IIA. Muscle fiber typing of other muscles in the quadriceps yielded three fiber types but little size variation.

Sections of M. vastus lateralis revealed atrophy of type I fibers and hypertrophy of type IIB fibers. The primary lesion of the weaver syndrome is atrophy of type I fibers which are essential for maintaining normal posture. The presence of degeneration in absence of regeneration, classifies the weaver syndrome as a muscular dystrophy.